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Certifying Officer

PROVISIONAL APPLICATION COVER SHEET **Mail St p Provisional Patent Application**

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Docket Number 07588.6000		Type a plus sign (+) inside this box ⊟	+

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TITLE OF INVENTION (500 characters max)

THERAPEUTIC MICROFOAM

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification	42 Pages	☐ Small Entity Statement
☐ Drawing(s)	0 Sheets 0 Figures	☐ Other (specify)

METHOD OF PAYMENT (check one)

☒	A check or money order is enclosed to cover the Provisional filing fees	PROVISIONAL FILING FEE
☒	The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number 06-0916.	☒ $160.00 ☐ $80.00 (small entity)

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE Bryan C. Diner Date February 10, 2004

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☐ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

Therapeutic Microfoam

The present invention relates to the generation of foam comprising a sclerosing material, particularly a sclerosing solution, which is suitable for use in the treatment of various medical conditions involving blood vessels, particularly varicose veins and other disorders involving venous malformation.

Sclerosis of varicose veins is based on the injection into the veins of liquid sclerosant substances which, by *inter alia* causing a localised inflammatory reaction, favour the elimination of these abnormal veins. Until recently, sclerotherapy was a technique selected in cases of small and medium calibre varicose veins, those with diameters equal to or greater than 7 mm being treated by surgery.

An injectable microfoam suitable for therapeutic use, on larger veins in particular, has now been developed and is described in EP-A-0656203 and US 5676962 (Cabrera & Cabrera), incorporated herein by reference.

Prior to the priority date of these patents it had been known for many years that injection of liquid sclerosant into varicose veins, especially smaller varicose veins, could be effective. It had also been known for many years to inject a small quantity of air into a vein prior to injecting sclerosing liquid, the objective being to displace blood from the vein to avoid the sclerosing agent being diluted too quickly. A development of this technique was to make a loose foam or froth and to inject this instead of pure air, prior to injection of the sclerosant liquid. These techniques, known as "air block" and developed by Orbach, were generally only effective for treating smaller veins.

In addition there had been disclosures of finer foams for treatment of smaller varicose veins (Fluckiger references cited below), or a combined procedure using both surgery and foam for treatment of the entire long saphenous vein: Mayer; Brucke: *"The Aetiology and Treatment of Varicosities of the Lower Extremities", Chirurgische Praxis, 521-528, 1957.*

All of these prior disclosures of foam/froth treatment describe the preparation of the foam/froth with air as the gaseous component. None of the documents mentions the air in the injected foam giving rise to serious problems. One reference mentions an apparently short lived air embolism: P.Fluckiger: *"Non-surgical retrograde sclerosis of varicose veins with Varsyl foam", Schweizerische Medizinische Wochenschrift No.48, pp1368-1370 (1956).* In this article, the author indicates that he reduced the volume of foam administered to 10ml from 15ml as a result of a patient experiencing chest pain on standing immediately after treatment with 15ml of foam. In a later lecture, the same author indicates that he has in fact subsequently used 15ml foam without noting ill effects: *lecture dated 1962 entitled "A contribution to techniques for outpatient treatment of varicose veins" delivered to the Hamburg Dermatological Society.* The reference by Mayer and Brucke cited above appears to describe the use of as much as 50ml of air foam and does not mention any problems.

It is known that rapid intravenous injection of a large quantity of air, as opposed to air foam, can lead to air embolism which may be fatal. In spite of this practitioners of the air block and foam techniques described above do not report that the volumes of air involved in their techniques were sufficient to cause serious problems.

The first teaching that potential issues with intravenous injection of a foam product made with air are serious enough to warrant change is to be found in the Cabrera patent references mentioned above. These documents indicate that the prior air based techniques are "dangerous owing to the side effects of atmospheric nitrogen which is only slightly soluble in blood", though it is not mentioned exactly what the dangers are nor what volumes or rates of injection of air or nitrogen gas give rise to these dangers.

The Cabreras proposed the use of a microfoam, that is to say a foam with microscopically small bubbles, for injection into varicose veins. The use of a microfoam, as opposed to larger bubbled foam or froth, gives rise to many advantages in terms of controllability and ability to displace blood in even the largest varicose veins, allowing treatment of virtually all varicose veins without recourse to surgery.

In addition to being the first to propose a microfoam as opposed to a larger bubbled foam, and to propose treatment of even the largest veins without surgery, the Cabreras also proposed that the microfoam be made with oxygen or a mixture of carbon dioxide and oxygen. Carbon dioxide is very soluble in water (and hence blood); oxygen is not very soluble in water but is taken up relatively rapidly by haemoglobin in blood. Microfoams made solely with carbon dioxide, or other highly water-soluble gases, tend to be very unstable and do not last long enough to be usable. Oxygen microfoams do not have this problem, but the injection of oxygen gas has been reported to be dangerous and, in fact, has been said to be almost as dangerous as air when injected into the venous system. See, for example, Moore & Braselton *"Injections of Air and carbon Dioxide into a Pulmonary Vein", Annals of Surgery, Vol 112, 1940, pp 212-218.*

In the context of this background, the Cabreras' contribution can be seen to be highly innovative in a number of respects – appreciating against the prevailing thinking at the time (i) the potential of a sclerosant microfoam, (ii) the need for soluble gases, (iii) the use of oxygen which does not degrade the microfoam yet is taken up by blood, (iv) the safety of oxygen but also (v) the possibility of incorporating a percentage of highly soluble carbon dioxide.

The air block technique had largely fallen out of favour by the 1980s and the other foam techniques mentioned above were virtually unheard-of. Since publication of the Cabreras' microfoam technique in the mid 1990s, however, many practitioners have adopted foam both in Europe and the USA. At the recent worldwide conference of phlebologists in San Diego in August 2003, approximately one third of the two hundred and fifty or so papers which were presented concerned foam treatment.

Almost without exception, practitioners using sclerosing foam today make it with air. Opinion varies as to how much foam should be injected – some advocate as little as 5ml whilst others are prepared to inject more. The use of pure nitrogen is taught in at least one reference.

The Cabreras' microfoam is prepared extemporaneously in the clinic immediately prior to use. The preparation involves beating sclerosant solution with a small brush

rotated at high speed by a motor, under a cover which is connected to a source of oxygen or oxygen and carbon dioxide. Most practitioners who have followed the Cabreras use an alternative technique for extemporaneous preparation of foam which involves passing sclerosant solution and air repeatedly between two connected syringes. Another alternative is a syringe with a second plunger with holes in its face and which is independently movable in the syringe barrel to froth a liquid and gas mixture in the syringe. Both of these latter types of procedure are somewhat inconvenient and allow for variation of the foam composition depending upon the person preparing it: gas content, bubble size, density and stability all require attention. These techniques require a high degree of care and knowledge that may be difficult to replicate under pressure, i.e. when time available to prepare the foam is short.

A product which aims essentially to reproduce the Cabreras' microfoam in a more convenient and easily reproducible way is currently being developed and is in clinical trials in Europe and the USA. This product is a pressurised canister system, in which the microfoam is produced by passing gas and sclerosant solution under pressure through a number of fine meshes. In the trials of this product the aim is to treat an entire long saphenous vein and its varicosed tributaries in a single treatment, which can mean injection of 25ml or even 50ml of foam.

WO 00/72821-A1 (BTG International Limited), incorporated herein by reference, describes the fundamental concepts underlying this canister product. The foam is produced by passing gas and sclerosant liquid through one or more meshes having small apertures measured in microns. Like the Cabrera patents, this document acknowledges the potential issues with air / nitrogen and seeks to reduce the levels of nitrogen in the foam. A preferred form of gas described in WO 00/72821-A1 comprises 50% vol/vol or more oxygen, the remainder being carbon dioxide, or carbon dioxide, nitrogen and trace gases in the proportion found in atmospheric air.

In a later patent application, WO 02/41872-A1 (BTG International Limited), incorporated herein by reference, the sclerosant liquid and an oxygen-rich physiologically acceptable blood dispersible gas are stored in separate containers until immediately prior to use, when the blood-dispersible gas is introduced into the

container holding the sclerosant liquid. The mixture of blood-dispersible gas and sclerosant liquid is then released, the components of the mixture interacting upon release of the mixture to form a sclerosing foam. In the system described in this patent application, a proportion of nitrogen (25%) is deliberately introduced into the polidocanol canister. After charging of the sclerosing liquid (polidocanol) can with oxygen from the higher pressure oxygen canister, the percentage of nitrogen is reduced to about 7 or 8%. It was believed that this level of nitrogen could be tolerated.

The present inventors are continuing to research clinical aspects of the injection of sclerosing microfoam as well as developing the canister microfoam product and putting it through clinical trials in Europe and the USA. It has always been the intention to develop a safe microfoam product which is as well defined as possible but whose specification has achievable tolerances. There are many parameters of a microfoam which may be varied. These include, without limitation: the chemical, its purity and the strength of the solution; the size of bubbles, or more accurately the distribution of sizes, the density (i.e. ratio of liquid to gas), the longevity of the microfoam (measured in terms of "half life", or the time taken for half the foam to revert to liquid) and the gas mixture.

Nitrogen, which makes up approximately 80% of air, is difficult as a practical matter to exclude totally from a foam. This is true whether the foam is made using a canister system, in which case nitrogen tends to creep into the canister during manufacture, or using either of the syringe techniques or the Cabreras' rotating brush technique, or indeed any of a number of other less common techniques which have been developed since the Cabreras' disclosure of microfoam.

In a two syringe technique the likely method for introducing the gas component, if a foam were to be made with a gas other then air, would be to connect one syringe to a pressurised source of gas, then disconnect and reconnect it to another syringe containing sclerosant. In this sort of technique, the two syringes are pumped to create foam and then the foam-filled syringe separated. The potential for ingress of a small percentage of air/nitrogen during this process is obvious. Similarly, even with the

Cabreras' technique, it may be difficult to exclude 100% of air/nitrogen from the environment in which the foam is prepared.

One of the objectives of the foam product being developed by the inventors is to treat an entire greater saphenous vein together with major varicose tributaries in a human patient with one injection. This requires up to 25ml, 30ml or possibly even 50ml of microfoam. Currently, the most conservative users of air foam inject a maximum of 5ml into the venous system, apparently without observing any deleterious effects. The inventors therefore reasoned that an equivalent amount of nitrogen in a relatively large dose of microfoam needed to treat the entire saphenous vein should also be safe. They therefore used this as a starting point: 5ml of air with 80% nitrogen will contain 4ml nitrogen; a corresponding proportion of nitrogen in, say, 50ml of low nitrogen foam would be around 8%.

Until recently, its has been believed by the inventors that a foam with approximately 8% nitrogen would be acceptable from a safety standpoint and that this percentage represented an easily achievable tolerance for nitrogen levels in the foam specification. Accepting this level of nitrogen also has the advantage that a small quantity of nitrogen could be introduced deliberately into the polidocanol canister to reduce the adverse effects of the highly soluble carbon dioxide on the foam stability (as discussed above). This microfoam and a system for making it is described in WO 02/41872-A1, referred to above.

As discussed above, apart from the above mentioned patent publications, the published art on foam treatment of varicose veins mentions little if any danger from injecting air foam up to 15ml. The only event noted by Fluckiger was temporary chest pain. The above mentioned patent publications which mention dangers with nitrogen are silent regarding the amount of nitrogen which would be dangerous and what damaging effects it may cause. A great many practitioners are currently using air based foam, though some restrict the quantity injected to 5ml. The inventors have been involved in a 650 patient multi-centre European phase III clinical trial of the canister product described above which contains 7-8% nitrogen; no serious adverse events associated with the gas component of the foam were noted.

Now, further research in connection with the clinical trials of the canister system described above has revealed the presence of large numbers of bubbles in the heart, some of which endure for a significant period of time. Ultrasound monitoring of the heart during treatment of patients in this trial has revealed many bubbles on the right side of the heart and in associated blood vessels. Since microfoam is injected into the venous circulation, i.e. that connected to the right side of the heart, it was expected that some bubbles on the right side of the heart would be observed. However, the number and persistence of the bubbles was surprising.

Furthermore, bubbles have been observed on the left side of the heart in a patient who was subsequently shown to have a minor septal defect, or patent foramen ovale ("PFO"), i.e. a hole in the heart. The patient reported experiencing a transient visual disturbance. This is significant because, once on the left side of the circulation, the bubbles can progress to the brain, where they may cause microinfarcts.

At present it is believed that screening all patients for even the most minor PFO is not really feasible for an elective procedure such as varicose vein treatment and may not even be possible. The techniques required would be fairly sophisticated and possibly quite invasive. Furthermore this would increase the time required for the procedure and preclude treatment of patients having such PFOs, of which it is believed there are significant numbers.

In the light of these unexpected findings, considerable further fundamental research has been carried out by the inventors.

Experiments using animal models have been carried out by the inventors and internationally recognised experts in their field have been commissioned to carry out detailed mathematical modelling of the behaviour of oxygen, carbon dioxide and nitrogen bubbles in blood. In vitro work to measure the absorption of gases in fresh human venous blood has also been carried out by the inventors. As a result it has become clear that, contrary to previous thinking by the inventors, and in stark contrast to the thinking of almost every practitioner currently preparing extemporaneous microfoam for use in varicose vein treatment, even the smallest volume of nitrogen is highly significant in causing persistent bubbles.

The inventors have now determined that in order to produce a product suitable for administration to patients without the need for lengthy PFO screening methodology it is required to reduce the amount of nitrogen to upper levels previously unrecognised as necessary.

Further developments of the canister system described in WO00/72821-A1 and WO02/41872-A1 have been devised, specifically raising the percentage of carbon dioxide in the foam and reducing the nitrogen present in the foam to near zero. To compensate for the deleterious effects of the highly soluble carbon dioxide, the size of the apertures in the mesh has been reduced to 5 microns from 20 microns. Canisters of this design have been made in reasonably large numbers for testing. Initially, double canister systems as described above were prepared by flushing the canisters with the desired gas before sealing and pressurising them. This product generated a foam with between 1% and 2% nitrogen. Further research has led the inventors to believe, however, that even this level is too high.

Recognising that there will always be impurity no matter what technique is adopted for making the microfoam, the inventors believe that a sclerosing microfoam having a percentage by volume of nitrogen gas within the range 0.01% and 0.8% is both clinically safe and consistently reproducible. It may be possible routinely to produce canisters with as little as 0.0001% nitrogen gas. Examples presented below illustrate the manufacture/preparation and also the clinical effects of such a microfoam.

The inventors also recognise that techniques such as those described above using syringes, together with a variety of other techniques for extemporaneous preparation of sclerosing foam which have been developed since the Cabreras disclosure, may have their place in the field of foam scleropathy. These techniques may well provide a less expensive option than a canister product. The inventors believe that it is possible to prepare foams having a very low percentage of nitrogen, as set out above, using these types of technique as well as using a canister system.

According to the present invention, a foam consists of a liquid and a gas phase wherein the liquid phase comprises a sclerosing agent and the gas phase at consists

of 0.0001% to 0.8% by volume gaseous nitrogen, the remainder being made up from other gas, preferably physiologically acceptable gas.

By "physiologically acceptable gas" is meant gases which are relatively readily absorbed by the blood or which can pass rapidly across the pulmonary gas exchange membranes. Specifically, oxygen, carbon dioxide, nitrous oxide and helium are contemplated. Other gases, which may or may not fall within the terms of the definition of physiologically acceptable gases, may be used at least in small quantities, e.g. xenon, argon, neon or others. Gases which are found only at trace concentrations in the atmosphere (such as those just mentioned) may be useful to incorporate in the formulation, e.g. at relatively low concentrations of between about 0.1% and 5%, in order to facilitate the detection of leaks.

The said other gas preferably consists essentially of oxygen. Another preferred possibility is for the other gas to consist essentially of oxygen and a minor proportion, preferably 40% or less of carbon dioxide, still more preferably 30% or less of carbon dioxide. In these cases, between 0.1% and 5% of the other gas may be constituted by gases which are only found at trace levels in the atmosphere, e.g. argon, helium, xenon, neon.

For the purpose of this application various other terms have the following definitions: A sclerosant liquid is a liquid that is capable of sclerosing blood vessels when injected into the vessel lumen and includes without limitation solutions of polidocanol, tetradecyl sulphate, ethanolamine oleate, sodium morrhuate, hypertonic glucosated or glucosaline solutions, chromated glycerol, iodated solutions. Scleropathy or sclerotherapy relates to the treatment of blood vessels to eliminate them. An aerosol is a dispersion of liquid in gas. A major proportion of a gas is over 50% volume/volume. A minor proportion of a gas is under 50% volume/volume. A minor amount of one liquid in another liquid is under 50% of the total volume. Atmospheric pressure and bar are 1000 mbar gauge. Half-life of a foam (including a microfoam) is the time taken for half the liquid in the foam to revert to unfoamed liquid phase.

Preferred ranges for the gaseous nitrogen volume at are 0.0001% to 0.75%, more preferably 0.7%, still more preferably 0.6%, optimally 0.5%. Although from a theoretical viewpoint it is desirable to eliminate as much nitrogen as possible, it is also understood that since we live in an atmosphere of 80% nitrogen there are difficulties in consistently making a foam with a very high degree of purity with regard to nitrogen gas. Accordingly, the lower end for the range of nitrogen impurity which is preferable (from the point of view of being easier and/or less expensive to manufacture) is 0.0005%, more preferably 0.001%, still more preferably 0.005%, 0.01%, 0.05%, 0.1%, 0.2%, 0.3% or 0.4%. As will be apparent from the examples below, each incremental increase in the lower end of the range may result in a purifying step being taken out of the manufacturing procedure, with resulting cost savings.

Also according to the invention is provided a canister system adapted to dispense a foam and whose contents consist of a liquid phase and a gas phase, wherein the liquid phase comprises a sclerosing agent and the gas phase consists of a minor proportion of nitrogen gas and a major proportion of other gas, preferably physiologically acceptable gas, such that the gas phase of a foam produced by the canister system consists of between 0.0001% and 0.8% nitrogen gas. The other possible ranges for the nitrogen gas component, as recited above, also apply.

It will be appreciated that the term "canister system" can mean either a single canister containing a liquid and a gas for dispensing to generate a foam, or a two canister arrangement as described above, where gas is stored in one canister and liquid, optionally together with gas, in another.

Preferably the said minor proportion of nitrogen gas in the canister is also 0.0001% to 0.8% by volume of the total gas volume in the canister, or optionally the other ranges recited above.

Preferably, the canister includes an element through which the liquid and gas contents pass in order to dispense foam. Preferably, this element has apertures of approximately 0.1 to 15micron diameter, more preferably 1-7micron, still more preferably about 5micron.

Another aspect of the present invention is a method for producing a microfoam suitable for use in scleropathy of blood vessels, particularly veins, characterized in that it comprises passing a mixture of gas and an aqueous sclerosant liquid through one or more passages having at least one cross-sectional dimension of from 0.1 to 15 μm, the ratio of gas to liquid being controlled such that a microfoam is produced having a density of between 0.07 g/mL to 0.19 g/mL and a half-life of at least 2.5 minutes.

Preferably, the said one or more passages have at least one cross-sectional dimension of from 1-7 micron, more preferably about 5 micron.

In accordance with the original specification (as set out in WO00/72821-A1), the microfoam is preferably such that 50% or more by number of its gas bubbles of 25 μm diameter and over are no more than 200 μm diameter. Again in accordance with the original specification in WO00/72821-A1, preferably the method provides a microfoam characterised in that at least 50% by number of its gas bubbles of 25 μm diameter and over are of no more than 150 μm diameter. More preferably at least 95% of these gas bubbles by number are of no more than 280 μm diameter. Still more preferably at least 50% by number of these gas bubbles are of no more than 130 μm diameter and still more preferably at least 95% of these gas bubbles by number are of no more than 250 μm diameter.

Preferably the gas/liquid ratio in the mix is controlled such that the density of the microfoam is 0.09 g/mL to 0.16 g/mL, more preferably 0.11 g/mL to 0.14 g/mL. Preferably the microfoam has a half-life of at least 3 minutes. The half-life may be as high as 1 or 2 hours or more, but is preferably less than 60 minutes, more preferably less than 15 minutes and most preferably less than 10 minutes.

Half-life is conveniently measured by filling vessel with a known volume and weight of foam and allowing liquid from this to drain into a graduated vessel, the amount drained in a given time allowing calculation of half-life i.e. of conversion of microfoam back into its component liquid and gas phases. This is preferably carried

out at standard temperature and pressure, but in practice ambient clinic or laboratory conditions will suffice.

Preferably the mixture of gas and sclerosant liquid is in the form of an aerosol, a dispersion of bubbles in liquid or a macrofoam. By macrofoam is meant a foam that has gas bubbles that are measured in millimetres largest dimension, e.g. approx. 1 mm and over, and over such as can be produced by lightly agitating the two phases by shaking. Preferably the gas and liquid are in provided in the form of an aerosol where a source of pressurized gas and a means for mixing the two is provided to the point of use. It may be preferred that a macrofoam is first produced where the liquid and gas are brought together only at the point of use.

The ratio of gas to liquid used in the mixture is important in order to control the structure of the microfoam produced such that its stability is optimized for the procedure and the circumstances in which it is being carried out. For optimum foams it is preferred to mix 1 gram sclerosant liquid with from approximately 6.25 to 14.3 volumes (STP), more preferably 7 to 12 volumes (STP), of gas.

Preferably the gas comprises from 1% to 50% carbon dioxide, preferably from 10% to 40%, more preferably from 20% to 30%. Surprisingly, it has been found that by using a smaller aperture size for the mesh, foams having the specification set out in WO00/72821-A1 can be made with gas mixtures having higher proportions of carbon dioxide and correspondingly lower proportions of insoluble gases such as nitrogen. Carbon dioxide is a desirable component of the gas mixture due to its extreme solubility, greater than that of oxygen.

Also according to the invention a method for angiologic treatment comprises injecting an effective amount of a sclerosing foam whose gaseous component consists of between 0.0001% and 0.8% by volume gaseous nitrogen, the balance being other gas, preferably physiologically acceptable gas. The other possible ranges recited above for the percentage of nitrogen apply and the options for the other gases recited above apply.

Preferably the method of treatment comprises the injection of 10ml to 50ml of foam in a single injection, preferably 15ml to 50ml, more preferably 20ml to 50ml, still more preferably 30ml to 50ml of foam.

According to the invention a method of treatment of the human greater saphenous vein comprises treating substantially the entire greater saphenous vein of one leg with a single injection of foam as described above.

According to the invention a method of treatment of a blood vessel of diameter 7mm or greater so as to cause damage to the endothelium of the vessel comprises injecting foam as described above.

A further factor in the inventors' developing understanding of the behaviour in blood of bubbles comprising soluble gases is the phenomenon of nitrogen diffusing out of blood and adjacent tissues and into the bubbles due to a difference in the partial pressure of nitrogen in the bubbles as compared with that in the surrounding blood and tissues. This phenomenon will generally only occur when the partial pressure of nitrogen in the bubble is lower than that in the surrounding blood and tissues.

It appears that carbon dioxide, and to a lesser extent oxygen, will diffuse out of the bubble and go into solution in the surrounding blood relatively very quickly, so that the bubble will quite quickly reach a point where the partial pressure of nitrogen in the bubble will be higher than that in the surrounding blood and tissues and, ultimately, the bubble will become substantially pure nitrogen. As soon as the nitrogen partial pressure gradient is reversed, nitrogen will come out of the bubble and into solution in the blood, though this will happen relatively slowly because of the low solubility of nitrogen. This phenomenon will also be influenced by increasing saturation of the surrounding blood with nitrogen, if this occurs to a significant extent. This phenomenon potentially affects the partial pressure gradient of nitrogen in the blood and may also mean that a limit for dissolution of nitrogen is reached if the surrounding blood becomes fully saturated with nitrogen.

It is not at present understood to what extent localised saturation of blood with nitrogen is a factor in the dissolution of the bubbles in a dispersing microfoam. Since

the bloodstream in constant motion, however, it is assumed that this effect will only ever be transient and will not unduly affect the overall picture of nitrogen dissolution.

It appears that the initial phase of rapid dissolution of carbon dioxide and/or oxygen is critical: the shorter this period, the smaller the volume of nitrogen which is able to diffuse into the bubble.

The inventors have conceived of two possible ways for eliminating residual bubbles or reducing them in size and/or number (apart from reducing the initial quantity of nitrogen in the gas phase of the foam). The first of these is to make the bubbles as small as is practical. The smaller the bubble, the faster the carbon dioxide and/or oxygen will dissolve out of the bubble and therefore the shorter the time available for nitrogen from the blood to diffuse into the bubble before the partial pressure gradient for nitrogen reverses in favour of nitrogen diffusing out of the bubble.

The second possibility is that of the patient breathing oxygen or air enriched with oxygen, which has the effect of increasing the oxygen partial pressure in the blood at the expense of the nitrogen partial pressure. This technique is known in the fields of diving and space exploration, where it has been used to reduce the risk of the "bends", i.e. the tendency on depressurisation for nitrogen to come out of solution in body tissues (as opposed to the blood in blood vessels which is what we are concerned with here). As far as the inventors are aware, it has never previously been proposed to use this technique in connection with injecting gases into the vascular system.

According to an aspect of the invention a sclerosant microfoam is composed of bubbles of which, ignoring bubbles of 1 micron or less diameter, 95% or more are of 150micron diameter or less and 50% or more are of 100micron diameter or less. Preferably, 95% or more of the bubbles are of 100micron diameter or less and 50% or more of the bubbles are of 50micron diameter or less. More preferably, 95% or more of the bubbles are of 75micron diameter or less and 50% or more of the bubbles are of 30micron diameter or less. Still more preferably, 95% or more of the bubbles are of 60micron diameter or less and 70% or more of the bubbles are of 30micron

diameter or less. Examples are presented below showing how foams with these sorts of bubble distributions have been made.

These very small bubble foams have only to date been obtained by the inventors by having a relatively dense formulation of the order of 0.3 to 0.5 g/ml, with a relatively high ratio of liquid to gas. Such a wet microfoam is still considerably less dense than blood and therefore will be buoyant when in a vein full of blood. It is speculated that this buoyant characteristic may to some extent be responsible for the advantageous behaviour of microfoam in the vascular system in terms of displacing blood. However, the dense microfoams produced to date by the inventors behave essentially as a liquid in terms of their rheological properties – they are not "stiff".

It is not impossible that these dense but somewhat fluid foams may have a sufficiently good therapeutic effect to be useful and may also eliminate or reduce the residual gas problem. However, it is probable that the rheological properties of the foam in blood are important, and that a "stiff" foam is desirable effectively to displace blood and thus allow consistent, uniform application of the active to the interior of the vessel wall. For this reason it may be desirable to add a further ingredient to the foam in order to increase its stiffness/viscosity, either by adding a viscosity-enhancing additive to the formulation or by adding an agent which increases the foaming capacity of the formulation.

Such ingredients could be, without limitation, Polysorbate 20, Polysorbate 80 or Polygeline (as suggested in the Cabrera patents cited above). Alternatively, glycerol may be added.

A foam with a bubble size distribution falling within the definitions set out above may be created by passing gas and liquid repeatedly through a fine mesh, e.g. a 5 micron mesh. Repeated passages through the mesh reduce the bubble size, though there appears to be a limit on this.

It is envisaged that other known techniques for agitating a gas and liquid mixture at high energy could be applied to make even finer bubbles. For example sonic or ultrasonic agitation of a mixing stream of gas and liquid could be used, or

alternatively a mixture of beating the gas and liquid by mechanical means, supplemented by the application of sonic or ultrasonic energy.

The inventors have also prepared a foam having an average bubble size in the range 50micron to 80micron by adapting a canister to alter the ratio of liquid and gas being passed through a mesh.

A further aspect of the invention is a pressurised canister product adapted to dispense a sterile gas and sclerosing liquid mixture in predetermined proportions into a syringe, as a solution to some of the issues with extemporaneous preparation of foam. Thus a pressurised canister is provided – which may be of any suitable material such as anodised aluminium or even glass – containing sterile gas and sclerosing liquid and arranged to dispense the correct volume of liquid and gas into a syringe. It is envisaged that the canister would contain sterile gas with a very low nitrogen concentration etc. as defined above. The canister may have a pierceable septum for puncturing with a hypodermic needle, or it may have a break seal which is arranged to be broken by insertion of a syringe luer nozzle.

In the latter case, a syringe luer nozzle could be inserted into the canister in a sealing fashion, with the syringe nozzle pointing upwards. Liquid in the canister would be dispensed first under pressure, followed by equalisation of the pressure in the canister and syringe. The pressure and volume of gas in the canister could of course be arranged so that the correct proportions of gas and liquid are dispensed. Alternatively, the canister could be provided with an internal dip tube so that the same effect is achieved with the canister in an upright orientation.

Also according to the invention is provided a method of preparing a sclerosing foam which includes the step of cooling the ingredients of the foam to a sub-ambient temperature prior to generation of the foam. A suitable temperature range might be 0 to 15 degrees Celsius, preferably 0 to 10 degrees, more preferably 3 to 7 degrees. Decreasing temperature increases liquid viscosity and, in this way, the inventors believe the half life of the foam could be extended. Since, during decay of a foam, the bubble size tends to increase, this methodology may help reduce the average size of bubbles over time in the body and thereby reduce residual bubbles.

Also according to the invention, and in line with the reasoning presented earlier, a method of angiologic treatment of a patient comprises causing the patient to breathe oxygen gas or oxygen-enriched air for a predefined period prior to injection of foam as described above. Preferably the predefined period is 1 to 60 minutes, more preferably 1-20 minutes, more preferably 5-10 minutes.

The following examples are provided in support of the inventive concepts described herein.

Example 1

10 patients were treated for varicose veins by injection of microfoam made with 1% polidocanol solution and a gas mix consisting essentially of 7-8% nitrogen and the remainder carbon dioxide (about 22%) and oxygen (about 70%).

The procedure involved the injection of up to 30ml of microfoam (25.5ml gas) into the thigh section of the greater saphenous vein. 4-chamber cardiac ultrasound examinations were conducted on all the patients to test for bubbles reaching the heart. Bubbles were observed in the right atria and ventricles of all 10 patients examined. In general, bubbles appeared several minutes following injection of the foam and continued until the ultrasound recording was stopped about 40 minutes after injection.

In one patient, microbubbles were observed in the left atrium and ventricle. This patient was subsequently confirmed to have a patent foramen ovale.

Example 2

The objective of this experiment was to investigate the nature of the residual bubbles that pass into the heart following injection into the saphenous vein of polidocanol microfoam made with different gas mixtures.

An anaesthetised female hound dog weighing 26 kg was injected with microfoam containing polidocanol formulated with varying gas mixes. Residual bubbles were monitored in the pulmonary artery using transoesophageal echocardiogram (TEE). Residual bubbles visualised on TEE were sampled from the pulmonary artery through a wide-bore catheter. These blood samples were analysed for the presence of residual bubbles using light microscopy and ultrasound.

Three different compositions of foam were used, as follows:

A. 1% polidocanol and air

B. 1% polidocanol and a gas mix consisting of 7-8% nitrogen and the remainder carbon dioxide and oxygen

C. 1% polidocanol solution and a gas mix comprising less than 1% nitrogen and the remainder carbon dioxide and oxygen.

The TEE output was videotaped and subsequently analysed. For all three compositions, bubbles reached the pulmonary artery in sufficient quantity to cause a substantially opaque image. It is believed that the threshold bubble density required to produce such an image as quite low, and therefore this image in itself did not provide useful data. The time taken for the occluded image to revert to a steady state background image was believed to be approximately indicative of the length of time taken for all or most the bubbles to have dissolved into the bloodstream. The TEE was very sensitive (showing activity even when saline was injected as a control); for this reason exact end points were difficult to determine. However, the following estimates have been made of the time period from opacification of the image to decay down to a background level.

A. 4 minutes

B. 2 minutes

C. 20 seconds.

In addition to the TEE analysis, observations were made of samples of blood drawn from the pulmonary artery for each foam during the period when the TEE image was substantially opaque. The results of these observations were as follows.

A. As soon as the sample was taken, a considerable volume of bubbles was observed in the syringe. When the syringe was held with its longitudinal axis horizontal, a continuous strip of bubbles was observed extending substantially the full length of the 20ml syringe.

B. Initially on taking the sample no bubbles were observed in the syringe, but after a few seconds, with the syringe in the horizontal position, a line of bubbles appeared which was thinner than the line observed for foam A.

C. After taking the sample and holding the syringe in the horizontal position, no bubbles were observed for a period of a minute or more. Gradually, a thin line of bubbles began to appear along the top of the syringe.

It was not possible to measure the bubbles, but they appeared to be smaller for composition C than for composition B, with the bubbles from composition B in turn smaller than those from composition A.

Example 3

In vitro experiments were conducted to determine the absorption of foam made with different gases in fresh human venous blood.

A 20ml polypropylene syringe barrel was prepared by puncturing its side wall with a relatively large hypodermic needle to make a hole approximately 1mm in diameter. This hole was then covered by securing a piece of clear flexible vinyl sheet over it with clear adhesive tape. A small magnetic stirrer element was introduced into the syringe barrel and the plunger then replaced. 20ml of human venous blood was then with withdrawn in the usual manner from a human subject using the specially prepared syringe fitted with a hypodermic needle.

The hypodermic needle was removed and the syringe then placed on a magnetic stirrer unit so that the magnetic element in the syringe thoroughly agitated the blood. The Luer nozzle of the syringe was then connected to a 50cm piece of manometer tubing which was arranged horizontally and left open at one end. The manometer tubing was secured against a scale.

A 0.5ml measuring syringe with a fine pre-fitted needle was then filled with microfoam made from 1% polidocanol solution and air. The density of the foam was 0.13g/ml (±0.03g/ml), the liquid component making up approximately 13% of the total volume of foam (± 3%).

The needle of the 0.5ml syringe was then introduced through the vinyl sheet on the side wall of the 20ml syringe. A small volume of blood was found to have entered the manometer tubing and the position of the distal end of this column of blood was noted against the scale. The 0.5ml aliquot of microfoam was then injected quickly and simultaneously a timer started (t_0). As the foam displaced blood in the 20ml syringe, the column of blood from the 20ml syringe was displaced into the manometer tubing and the distance along the tubing reached by the distal end of the blood column was noted against the scale. The scale itself comprised spaced marker lines equally spaced at about 1cm intervals. It was determined that a distance of 45 intervals on this scale corresponded to an internal volume of in the manometer tubing of approximately 0.5ml.

As the gas in the microfoam started to be absorbed by the blood, the blood in the manometer tubing started to recede back towards the syringe. After the column appeared to have stopped moving, the timer was stopped (t_F). The position of the distal end was again noted.

This experiment was then repeated for a foam of the same density but made with oxygen gas ("medical grade" purity – 99.5% minimum).

The experiment was repeated again but this time oxygen gas from a cylinder of medical grade oxygen was introduced directly into the 0.5ml syringe instead of microfoam.

The results of these three tests ate presented below in Table 1

Table 1

Test	Foam/gas	Start position of blood ("x")	Position of blood at t_0 ("y")	t_F (seconds)	Position of blood at t_F ("z")	Absorbed volume at t_F (ml) $\frac{0.5(y-z)}{(y-x)}$	Liquid Volume in foam (ml)	Unabsorbed gas	
								ml	%
1	Air foam	2	47	80*	40	0.08	0.13 x 0.5 = 0.07	0.35	81%
2	Oxygen foam	4	48	140	11	0.42	0.13 x 0.5 = 0.07	0.01	2%
3	Oxygen gas	2	47	140	5.5	0.46	nil	0.04	8%

*No further movement of the blood column was observed after 80 seconds.

The experimental error in this example is unfortunately too great to conclude whether there is or is not a residual volume of gas for the oxygen gas or oxygen foam, although clearly the great majority at least of the gas is absorbed. There will have been a small percentage of nitrogen in the gas, from the oxygen cylinder which is only 99.5% pure, and possibly also introduced during the experiment. Diffusion of nitrogen into the bubbles from the blood is also a possibility, as discussed above, and some nitrogen may have been introduced inadvertently during the procedure.

In this experiment, the air foam test was only observed for a few minutes after t_F. However, further experiments have been conducted by the inventors, the results of which are not formally recorded here, involving foam with a percentage of nitrogen. A 20ml syringe of fresh human venous blood, as in the above experiments, was injected with a 0.5ml aliquot of a foam containing a percentage of nitrogen. The contents of the syringe were agitated as above and a period of 24 hours allowed to elapse. An easily visible volume of bubbles remained in the syringe.

Example 4 - preparation of ultra-low nitrogen canister

An anodised aluminium canister with an open top was filled with water. The canister was then immersed in a bath of water and inverted. A line from a pressurised cylinder of oxygen gas was then introduced into the water bath and the supply of oxygen turned on, thereby flushing the line of any air. A canister head assembly comprising a valve, dip tube and mesh stack unit was then immersed in the water bath and connected to the oxygen line for a few seconds to purge air from the assembly.

The oxygen line was then introduced into the inverted canister until all water had been displaced from the canister. The line was then removed from the canister and the previously purged head assembly quickly clamped over the top of the canister thereby sealing the canister. The canister was then removed from the water bath with the head assembly still clamped against it; the head assembly was then secured to the canister using a standard crimping technique.

The canister was then pressurised to about 8 bar absolute pressure by connecting the canister valve to a regulated oxygen line for 1 minute. The pressure as then relieved by opening the valve until the pressure in the canister was just above 1 bar absolute; a pressure gauge was applied to the valve intermittently during the pressure release operation to ensure that the canister pressure did not drop all the way down to 1 bar absolute. This was done to avoid the possibility of atmospheric air seeping into the canister.

The canister was then pressurised again up to about 8 bar absolute and the pressure release operation repeated. This process was then repeated a third time, with the final canister pressure being from 1.1 to 1.2 bar absolute.

18ml 1% polidocanol solution was then introduced through the canister valve using a syringe with all air pockets, including any air in the luer nozzle, removed. The canister valve was then connected to a carbon dioxide cylinder and pressurised to 2.2 bar absolute. Then the oxygen line was again connected to the valve and the pressure increased to 3.6 bar absolute.

Table 2 below shows the expected result from the oxygen pressurising and depressurising cycles, assuming 100% pure oxygen in the cylinder and assuming that despite the precautions taken 1% of the gas in the canister after the initial oxygen filling procedure is nitrogen. The worst case is assumed for the canister pressure values, namely 1.2 bar absolute ("bara") and 7.6 bara.

Table 2

	N2 partial pressure (bara)	Canister pressure (bara)	%N2
Start	0.012	1.2	1%
1st cycle	0.012	7.6	0.16%
	0.00189	1.2	0.16%
2nd cycle	0.00189	7.6	0.02%
	0.000299	1.2	0.02%
3rd cycle	0.000299	7.6	0.00%
	0.0000472	1.2	0.00%

As can be seen the percentage of nitrogen drops down to zero, calculated to two decimal places, after the three oxygen pressure/release cycles.

The oxygen cylinder used in the above process was a standard medical grade oxygen cylinder supplied by B.O.C. and specified at 99.5% or greater purity. The carbon dioxide cylinder used was so called "CP Grade" from B.O.C. which has a purity level of 99.995%.

Working to two decimal places, the impurity (which will be mainly nitrogen) arising from the initial filling procedure should be reduced to zero after three pressure/release cycles. Similarly the impurity level in the canister from the carbon dioxide cylinder can be considered zero to two decimal places, since the purity of the source was

99.995% and only approximately one third of the gas in the finished canister was carbon dioxide.

The inventors will perform further experiments along the above lines using oxygen and carbon dioxide sources of higher purity. The following cylinder oxygen is readily available from B.O.C.:

- "Medical grade" 99.5% purity (as used in the above procedure)
- "Zero grade" 99.6% purity
- "N5.0 grade" 99.999% purity
- "N5.5 grade" 99.9995% purity
- "N6.0 grade" 99.9999% purity

In each case the impurity is mainly nitrogen.

The following cylinder carbon dioxide products are readily available from B.O.C. They have the following specifications:

- "CP grade N4.5" 99.995% purity (as used in the above procedure)
- "Research grade N5.0" 99.999% purity.

It will be appreciated that repeating the procedure described above using "Zero grade" oxygen would result in a finished canister having maximum impurity (which will be mainly nitrogen) of 0.4%.

Of course the number of pressure/release cycles may be increased in order further to reduce the theoretical maximum impurity if the oxygen and carbon dioxide sources were 100% pure. It is a simple calculation to show the number of cycles necessary to reduce the maximum percentage impurity level to zero, calculated to 3, 4 or 5 decimal places. Provided the canister pressure never drops to or below 1 bar absolute and provided the lines from the oxygen and carbon dioxide cylinders are flushed through with gas prior to attachment to the canister valve, there is no reason to assume that any significant impurity will enter the canister during the pressure/release cycles.

A refinement of the procedure to reduce further any opportunity for impurity to enter would be to introduce the polidocanol solution immediately after initial flushing. In

this way, any air/nitrogen introduced with the polidocanol will be eliminated during the subsequent pressure/release cycles.

A further refinement of the technique might be to maintain the water bath in an agitated state using a magnetic stirrer, under a continuously refreshed oxygen atmosphere for 24 hours. In this way, any dissolved nitrogen in the water bath should be eliminated and replaced with dissolved oxygen. Filling the canister from this oxygenated water bath should, it is postulated, remove the water bath as a possible source of nitrogen impurity.

It is envisaged that five, ten, twenty or even 100 pressure/release cycles could be performed.

In this manner, using appropriate sources of oxygen and carbon dioxide as detailed above, it will be possible to make a canister charged with polidocanol and an oxygen and carbon dioxide mix having a percentage impurity of 0.005% or less (mainly nitrogen) using CP grade carbon dioxide or 0.001% or less using research grade carbon dioxide. It should also be possible to make a polidocanol and oxygen canister with a percentage impurity of nitrogen gas of 0.0001% or less using N6.0 grade oxygen.

It will of course be appreciated that the production of canisters in this way having a somewhat higher minimum nitrogen level is not difficult and may be achieved, for example, by reducing the number of pressure/release cycles.

It will also of course be appreciated that substitution of polidocanol by an alternative liquid component would be a trivial matter.

Example 5 – preparation of ultra-low nitrogen canister

The inventors are at present developing a procedure for large scale manufacture of ultra-low nitrogen canisters, using a similar methodology. In this procedure, two canisters are manufactured, one containing oxygen at 5.8 bar absolute and the other carbon dioxide and polidocanol solution at about 1.2 bar absolute. In use, the

CO2/polidocanol canister is pressurised immediately prior to use by connecting it to the oxygen canister. This is described in WO 02/41872-A1.

There is therefore a separate manufacturing procedure for the oxygen and carbon dioxide / polidocanol canisters. However, it will be apparent that either procedure is applicable to production of a single canister product containing polidocanol and oxygen, carbon dioxide or a mix of the two.

The procedure will be described first for an oxygen canister, which is simply an anodised aluminium canister with a standard valve assembly in the top. Prior to fitting the valve assembly, the canister is first flushed with oxygen gas by inserting an oxygen line into the open top of an upright cylinder for 10 seconds. The line is then withdrawn. At this stage not all the air will have been eliminated and it is believed that the nitrogen impurity level is around 5% or 6%; this has not been measured specifically, but has been deduced from the measured impurity level at a later stage in the procedure (see below). It is not believed that flushing the canister for a longer period would substantially change this value for nitrogen gas impurity.

The valve assembly is then loosely fitted and a filling head brought into engagement around the top of the canister and valve assembly so as to make a gas-tight seal against the canister wall. Connected to the filling head is a line for oxygen. The canister is then brought up to a pressure of approximately 5.5bar absolute (bara). Nitrogen gas impurity at this stage has been measured by standard gas chromatography techniques to be about 1%.

At one stage it was thought to be acceptable to have the nitrogen impurity level at around 1%, but following the results of the clinical trial (Example 1), it has been determined that a lower nitrogen content is desirable. For this reason, further steps have been added to the procedure, as follows.

Maintaining the seal between the canister and filling head, the contents of the canister are exhausted via the filling head until the pressure in the canister is just over 1 bara. As with Example 4 above, this is to prevent any potential ingress of atmospheric air through the seal.

Maintaining the seal between the canister and filling head, the pressure is then increased again to about 5.5 bara and again this pressure is released down to just over 1 bara. The canister is then brought up to its final pressure of 5.5bara ± 0.4 bara. At this stage, the nitrogen gas impurity measured by gas chromatography is about 0.2%.

It will be appreciated that each of the pressure/release cycles should reduce the impurity due to residual air/nitrogen by a factor of about 5 assuming no leakage. It is reasonable to assume no leakage since a positive pressure is always maintained in the canister. Assuming a 100% pure source of oxygen, the theoretical nitrogen impurity after these three pressure/release cycles should be around 0.05%. Since the measured nitrogen level is around 0.2%, there is apparently either impurity in the line or nitrogen is entering the sample during the measuring process. It can at least be concluded that the impurity level is 0.2% or better.

It will be appreciated that polidocanol solution, or any other liquid sclerosing agent, could be added into the canister during the above procedure and the standard valve and dip tube could be replaced with a unit including foam generating means such as a small aperture mesh. In the final step, the pressure in the canister may be brought up to whatever is required, e.g. around 3.5 bara. In this way, a final pressurised canister product containing sclerosant and substantially pure oxygen could be made.

At present, the effects, including possible oxidising effect, of storing polidocanol solution under pressurised oxygen are not fully understood. Therefore, it is preferred at present to have a two canister system in which the polidocanol solution is stored under carbon dioxide and/or nitrogen.

In previous versions of the product (as used in Example 1), the gas mix in the polidocanol canister was 25% nitrogen and 75% carbon dioxide. The nitrogen was present in order to reduce the deleterious effect of the highly soluble carbon dioxide on the stability of the foam. In order to minimise both the carbon dioxide and the nitrogen content of the foam, this canister was maintained at 0.5 bara. This meant that, when the canister was connected to the oxygen canister and the final pressure raised to about 3.5 bara, the nitrogen content reduced to around 7%.

It was then realised by the inventors that (1) the canister needed to be maintained at above atmospheric pressure to avoid the risk of contamination and (2) the percentage of nitrogen was too high. A new design of can was produced in which the microfoam generating mesh has smaller apertures – 5 micron instead of 20 micron. Although it was previously thought that differences in size at this level would not have a significant effect on the microfoam, it was in fact surprisingly found that this reduction in mesh pore size was just sufficient to compensate for the increased percentage of carbon dioxide which resulted from having substantially pure carbon dioxide in the canister and also from maintaining it at just over 1 bara instead of 0.5 bara.

Using a polidocanol canister of this design, and an oxygen canister as described above which is pressurised only once, the resulting microfoam had a nitrogen impurity of around 1-2%. However, it is now considered that this level is still too high.

The current procedure is to insert a carbon dioxide line into the open top of a metal anodised canister for 10 seconds. The line is then withdrawn. At this stage not all the air will have been eliminated and it is believed that the nitrogen impurity level is around 5% or 6%. It is not believed that flushing the canister for a longer period would substantially change this value for nitrogen gas impurity.

18ml of 1% polidocanol solution is then introduced into the canister, a carbon dioxide line reintroduced and the canister flushed again for a few seconds.

The head assembly, including dip tube, valve and microfoam generating mesh unit, is then loosely fitted and a filling head brought into engagement around the top of the canister and valve assembly so as to make a gas-tight seal against the canister wall. Connected to the filling head is a line for carbon dioxide. The canister is then brought up to its pressure of approximately 1.2 bara. Nitrogen gas impurity at this stage has not yet been measured but is expected to be in the region of 0.8%.

The final nitrogen impurity of a foam generated from the charged polidocanol canister after it has been connected to the oxygen canister to bring it up to about 3.5 bara, is given by:

$(0.8 \times 1.2 + 0.2 \times 2.3) / 3.5 = 0.4\%$

Example 6

A canister was prepared of the type described in WO00/72821-A1 having a dip tube and a standard valve assembly provided with a pair of small air inlet apertures, together with a mesh stack unit having a 5micron aperture size. The size of the apertures in the valve was enlarged slightly compared with the valve arrangement described in WO00/72821-A1 (which is designed to produce a foam of density between 1.1g/ml and 1.6g/ml). The purpose of this modification was to increase the proportion of liquid to gas in the mixture passing through the mash stack.

The canister was filled with 18ml of 1% polidocanol solution and pressurised with a mixture of oxygen, carbon dioxide and nitrogen. A foam was then dispensed.

This procedure was repeated for different sizes of valve aperture and a number of foams produced, all having the appearance of a white liquid and densities in the range 0.3 to 0.5g/ml. Bubble size analysis was performed for each of these foams, which showed the average bubble size in the region of 50 to 80micron diameter.

Example 7

The above experiment was repeated but with the length and diameter of the dip tube adjusted rather than the size of the apertures in the valve unit. It was necessary to increase the volume of liquid in the canister to ensure that the shortened dip tube reached the liquid level in the canister. It was possible to produce the same type of foam as described in Example 6 above.

Example 8

The inventors envisage reproducing the above experiments using a pure oxygen or oxygen and carbon dioxide formulation having nitrogen impurity levels as described above. The same techniques as those described in Examples 4 and 5 may be followed for producing very low levels of nitrogen impurity.

Example 9: Study to assess the effect on physical properties of microfoam from changes to the mesh material in the mesh stack

This study outlines the effect on microfoam properties of changing the shuttle mesh pore size from 20 microns to 5 microns, in combination with changes to the gas pressure and gas composition in the canister. The study dates from before the inventors' realisation that a nitrogen concentration of 0.8 or below was desirable. Its main purpose was to test whether use of a 5micron instead of a 20micron mesh will compensate for eliminating the 25% nitrogen which was previously deliberately incorporated into the polidocanol canister. The "100%" carbon dioxide and "100%" oxygen referred to in this and the following examples will in fact incorporate levels of nitrogen impurity and the final dual canister product discussed in these examples will probably produce as microfoam of about 1-2% nitrogen impurity.

Two different gas compositions were used. In one, the canister containing the 1% polidocanol solution and a 75%/25% atmosphere of CO_2/N_2 is evacuated to 0.5 bar absolute pressure, whilst the other canister is pressurised to 5.9 bar absolute with oxygen. In the other, the canister containing the 1% polidocanol solution is pressurised to 1.2 ± 0.1 bar absolute with 100% CO_2, whilst the other canister is pressurised to 5.8 ± 0.1 bar absolute with oxygen.

The objective of the study is to examine and compare results obtained using 5 micron and 20 micron shuttle meshes, for PD canister pressures of 0.5 bar absolute with the current gas atmosphere and for 1.2 bar absolute PD canister pressures with a 100% CO_2 as the filling gas.

Materials and Methods:

All sample preparation was performed in a laminar flow booth keeping exposure times to atmosphere to a minimum.

Shuttle units containing a stack of 4 nylon 6/6 woven meshes of 6 mm diameter in a class 100K cleanroom moulding facility were used. They differ in the following aspects shown in Table 3 below.

Table 3. Physical characteristics of the 20 μm and 5 μm meshes compared

Mesh Type	Thickness (μm)	Pore size (μm)	Open Area (% area of pores)	Thread diameter (μm)
5 μm	100	5	1	37
20 μm	55	20	14	34

Bioreliance Ltd, Stirling, Scotland, U.K., made the 1% polidocanol solution for the study under controlled conditions to the formula in Table 4.

Table 4. Composition of the 1% polidocanol solution

Material	Quantities	
	% $^{w}/_{w}$	per 1000 g
Polidocanol	1.000	10.00 g
Ethanol 96% EP	4.200	42.00 g
Disodium Hydrogen Phosphate Dihydrate. EP	0.240	2.40 g
Potassium Di-hydrogen Phosphate. EP	0.085	0.85 g
0.1 M Sodium Hydroxide Solution [used for adjustment of pH: 7.2–7.5]	q.s.	q.s.
0.1 M Hydrochloric Acid	q.s.	q.s.
Water for injection. EP [used to adjust to final weight]	approx. 94.475 q.s. to 100.00%	approx. 944.75g q.s. to 1000.00 g
TOTAL:	100.00%	1000.00 g

The polidocanol solution was sterile filtered using a 0.2-micron filter before filling into clean glass screw top bottles.

Bi-can assemblies were prepared for testing to the specifications of gas mix and pressure in the polidocanol canister detailed in Table 5.

Table 5. Summary of PD canister preparation for each treatment group

Canister Label	Sample Type	Gas Composition	Gas Pressure (bar absolute)	Mesh Pore Size (μm)
C	Control 1	75% CO_2/25% N_2	0.5	20
D	Test 1	75% CO_2/25% N_2	0.5	5
A	Control 2	100% CO_2	1.2	20
B	Test 2	100% CO_2	1.2	5

The order of testing of the experimental series was important, in that changes in ambient laboratory temperature affect the half separation time results. Experiments progressed cyclically through the sample types rather than test all of one sample type, followed by all of another sample type. This minimised the effect of any drift in laboratory temperature throughout the experiments. The laboratory temperature was maintained as close to 20 °C as possible.

It was also essential that the temperature of the half separation time apparatus be allowed to fully equilibrate to ambient room temperature following cleaning and drying steps between successive experimental measurements.

Summary of Tests:

The tests and specifications performed on the bi-can units in this study are summarised in Table 6.

Table 6. Summary of tests and specifications

	TEST	SPECIFICATION
1	Appearance of Device	No corrosion of canisters or valves. Free from signs of leakage and external damage
2	Gas Pressure Polidocanol Canister Oxygen Canister	1.10 to 1.30 bar absolute for Type 2 samples 0.4 to 0.6 bar absolute for Type 1 samples 4.90 to 5.9 bar absolute
3.	Appearance of Micro-foam	Upon actuation, a white microfoam is produced. After the foam has settled, a clear and colourless liquid is observed.
4.	pH of Solution (collapsed microfoam)	6.6 to 7.5
5	Microfoam density	0.10 to 0.16 g/ml.
6	Microfoam Half Separation Time	150 to 240 seconds
7	Bubble Size (Diameter Distribution) < 30μm 30 μm to 280 μm 281 μm to 500 μm > 500μm	 ≤ 20.0% ≥ 75.0% ≤ 5.0% None
8	Particulates (Visible) and Sub-Visible)	Complies with Ph. Eur.
9	Particulates (Sub-Visible)	The collapsed microfoam contains not more than 1000 particles per ml ≥ 10 μm and not more than 100 particles ≥ 25 μm per ml.
10	Polidocanol identification by GC method	GC pattern and retention times to be equivalent to reference preparation
11	Polidocanol Assay	0.90 to 1.10% w/w
12	Related Substances	No single identified impurity >0.20% area. No single unidentified impurity >0.10% area. Total impurities ≤ 4.0% area

Results:

Results of the tests described in Table 6 on bi-cans prepared as described in Table 5 are summarised in the following paragraphs.

In all cases the appearance of the devices conformed to specification in that the device showed no corrosion of canisters or valves and were free from signs of leakage and external damage. Upon actuation of the charged PD canister a white microfoam was produced. After the foam had settled, a clear and colourless liquid was observed.

Microfoam from all devices conformed to density and half separation time specification. However, one unexpectedly low result was obtained (C1 canister 1) but an additional two devices were tested which behaved as expected. In spite of the low result, the average conformed to specification. In general, microfoam generated via the 5 μm shuttles had longer half separation times. Results are summarised in Table 7.

The average pH of the microfoam generated conformed to specification. However, microfoam produced from the 100% CO_2 canister were close to the lower limit of detection of the specification and in one instance (C2 canister 4) it was just below specification. Results summarised in Table 7.

The gas pressure in the oxygen cans and the polidocanol cans conformed to specification in all cases. In one instance (C1 canister 6) a slightly lower oxygen canister pressure than expected was recorded. Results are summarised here in Table 7.

Table 7. Table summarising the microfoam density, half separation time, pH and canister gas pressures

Test Condition	density (g/cm^3)	half life (sec)	pH	Gas pressure (bars abs) Oxygen	PD
Specification	0.10-0.16	150-240	6.6-7.5	4.9-5.9	0.4-0.6
100% CO_2, 1.2 Bar, 20 μm mesh					
Canister A1	0.12	164	6.7	5.6	1.1
Canister A2	0.13	150	6.7	5.5	1.1
Canister A3	0.13	153	6.6	5.8	1.1
Canister A4	0.15	154	6.5	5.5	1.1
Canister A5	0.13	154	6.7	5.6	1.1
Canister A6	0.15	154	6.5	5.6	1.1
Average	0.13	155	6.6	5.6	1.1
100% CO_2, 1.2 Bar, 5 μm mesh					
Canister B1	0.12	182	6.6	5.4	1.1
Canister B2	0.12	169	6.7	5.6	1.1
Canister B3	0.14	162	6.6	5.4	1.1
Canister B4	0.1	173	6.7	5.7	1.1
Canister B5	0.12	168	6.6	5.6	1.1
Canister B6	0.15	161	6.5	5.4	1.1
Average	0.13	169	6.6	5.5	1.1
75% CO_2/25% N_2, 0.5 Bar, 20 μm mesh					
Canister C1	0.14	157#	6.9	5.4	0.6
Canister C2	0.15	182	6.9	5.5	0.6
Canister C3	0.13	193	6.9	5.4	0.6
Canister C4	0.15	183	6.9	5.7	0.6
Canister C5	0.15	192	6.8	5.6	0.5
Canister C6	0.15	191	6.9	5.0	0.6
Canister C11	0.14	189	7.0	5.7	0.6
Canister C12	0.13	179	7.0	5.4	0.6

Average	0.14	183	6.9	5.5	0.6
75% CO_2/25% N_2, 0.5 Bar, 5 µm mesh					
Canister D1	0.15	203	6.9	5.4	0.6
Canister D2	0.12	209	7.0	5.6	0.6
Canister D3	0.16	198	6.8	5.6	0.6
Canister D4	0.12	205	6.9	5.7	0.6
Canister D5	0.12	208	6.9	5.4	0.6
Canister D6	0.15	205	6.9	5.6	0.6
Average	0.14	205	6.9	5.6	0.6

Bubble size distribution:

The average bubble size for all conditions was within specification with the exception of control 1 (C) where the >500 µm which averaged at one oversized bubble. Results are summarised here in Table 8.

Table 8. Table to summarise the bubble size distribution of microfoam generated

	Bubble Diameters (µm)			
	<30	30-280	281-500	>500
Specification	<=20%	>=80%	<=5%	None
100% CO_2, 1.2 Bar, 20 µm mesh				
Canister A1	8.2%	89.5%	2.3%	0
Canister A2	8.1%	89.7%	2.2%	0
Canister A3	7.9%	85.3%	6.8%	0
Canister A4	9.0%	88.3%	2.6%	1
Canister A5	7.9%	90.7%	1.5%	0
Canister A6	11.0%	88.1%	0.9%	0
Average	8.7%	88.6%	2.7%	0
100% CO_2, 1.2 Bar, 5 µm mesh				
Canister B1	7.8%	91.8%	0.4%	0
Canister B2	5.5%	94.2%	0.3%	0
Canister B3	8.6%	90.7%	0.7%	0
Canister B4	8.8%	91.1%	0.2%	0

Canister B5	7.7%	92.2%	0.0%	0
Canister B6	8.2%	91.3%	0.5%	0
Average	7.8%	91.9%	0.4%	0
75% CO_2/25% N_2, 0.5 Bar, 20 μm mesh				
Canister C1	8.9%	87.2%	3.9%	0
Canister C2	10.0%	89.3%	0.6%	0
Canister C3	8.9%	86.5%	4.5%	1
Canister C4	9.7%	87.7%	2.5%	4
Canister C5	10.7%	87.9%	1.5%	0
Canister C6	10.1%	88.0%	1.9%	0
Canister C11	9.6%	89.5%	1.0%	0
Canister C12	11.0%	87.6%	1.4%	0
Average	**9.7%**	**88.1%**	**2.5%**	**1.0**
75% CO_2/25% N_2, 0.5 Bar, 5 μm mesh				
Canister D1	7.8%	92.0%	0.2%	0
Canister D2	8.1%	91.4%	0.6%	0
Canister D3	10.9%	89.0%	0.1%	0
Canister D4	8.5%	91.2%	0.2%	0
Canister D5	8.8%	91.1%	0.1%	0
Canister D6	10.2%	89.8%	0.0%	0
Average	**9.0%**	**90.7%**	**0.2%**	**0**

Value from Control 1, canister 1 are not included in the average

Example 10. Further study to assess the effect on physical properties of microfoam from changes to the mesh material in the mesh stack

The study of Example 9 was repeated using a device in which the shuttle mesh pore size was 20 microns, 11 microns and 5 microns, in combination with changes to the gas pressure and gas composition in the canister. Bi-can assemblies were prepared for testing to the specifications of gas mix and pressure in the polidocanol canister detailed in Table 9.

Table 9. Summary of PD canister preparation for each treatment group

Sample Type	Gas Composition	Gas Pressure (bar absolute)	Mesh Pore Size (μm)
Control 1	75%CO_2/25% N_2	0.5	20
Control 2	100% CO_2	1.2	20
Test 2	100% CO_2	1.2	5
Test 3	100% CO_2	1.2	11

Various batches of the microfoam resulting from the test in which the shuttle mesh pore size was 11 microns had the following characteristics:

Bubble Diameters (micrometers)

<=30	>30-280	>280-500	>500
9.2%	90.2%	0.6%	0.0%
11.8%	88.2%	0.0%	0.0%
10.6%	89.4%	0.0%	0.0%
10.2%	89.8%	0.0%	0.0%
10.6%	89.1%	0.3%	0.0%
10.5%	89.4%	0.1%	0.0%

Bubble Diameters (micrometers) excluding those below 30 μm

>30-130	>30-280	>280<=500	>500
59.1%	99.4%	0.6%	0.0%
71.2%	100.0%	0.0%	0.0%
75.3%	100.0%	0.0%	0.0%
67.3%	100.0%	0.0%	0.0%
66.4%	99.7%	0.3%	0.0%
73.6%	99.9%	0.1%	0.0%

Density and Half Life

Density (g/cm3)	Half Life (Min)
0.12	180 sec
0.14	171 sec
0.14	175 sec
0.12	175 sec
0.13	177 sec
0.15	177 sec

Example embodiments of the invention include:

1. A foam consisting of a liquid phase and a gas phase wherein the liquid phase comprises a sclerosing agent and the gas phase consists of 0.0001% to 0.8% by volume gaseous nitrogen, the balance being other physiologically acceptable gas.

2. A foam as described in 1 wherein the gas phase consists of 0.001% to 0.8% gaseous nitrogen, preferably 0.01% to 0.8%, more preferably 0.01% to 0.7%, still more preferably 0.01% to 0.6%, the balance being other physiologically acceptable gas.

3. A foam as described in 1 or 2 wherein the said other physiologically acceptable gas is oxygen, carbon dioxide or a mixture thereof.

4. A canister, the contents of which consist of a liquid component and a gas component, maintained at above atmospheric pressure, wherein:
(a) the liquid comprises a sclerosing agent;
(b) the gas consists of 0.0001% to 0.8% by volume gaseous nitrogen, the balance being other physiologically acceptable gas.

5. A canister as described in 4 further comprising a foam generating element with one or more apertures formed therein, the aperture or apertures having maximum dimensions from 0.1 to 200micron, preferably 1 to 50micron, more preferably 2 to 30micron, still more preferably 3 to 10micron and optimally about 5micron.

6. A canister as described in 5 wherein the aperture or apertures have a maximum dimension of 3 to 10micron, and wherein the said balance consists of between 1

and 40% carbon dioxide gas, preferably between 10 and 30% carbon dioxide gas, and oxygen gas.

7. A canister as described in 4 or 5 wherein the said balance consists of oxygen.

8. A method of making a canister as described in 4, the method comprising:
(a) flushing the canister with a gas mixture essentially comprising physiological gas;
(b) introducing liquid sclerosing agent into the canister either before or after step (a);
(c) pressurising the canister to a first predetermined pressure above atmospheric pressure from a source of physiological gas whose level of nitrogen contamination is between 0.0001% and 0.5%.

9. A method as described in 8 further including the further step of partially exhausting the contents of the canister, followed by re-pressurising the canister from the same or a different source of physiologically acceptable gas whose level of nitrogen contamination is between 0.0001% and 0.5%.

10. A method as described in 9 wherein the pressure in the canister is maintained at or above the surrounding atmospheric pressure.

11. A method for angiologic treatment comprising injecting a foam as described in any of 1 to 3 into vessels to be treated.

12. A method as described in 11 for angiologic treatment of a patient, the method comprising having the patient breathe oxygen or an oxygen enriched atmosphere for a predetermined period prior to injecting the foam.

13. A method for phlebologic treatment comprising injecting a foam as described in any of 1 to 3 into vessels to be treated.

14. A method as described in 13 for phlebologic treatment of a patient, the method comprising having the patient breathe oxygen or an oxygen enriched atmosphere for a predetermined period prior to injecting the foam.

15. A method as described in 13 or 14 wherein substantially the entire greater saphenous vein of one leg of a human patient is treated by a single injection of foam.

16. A method as described in any of 11 to 15 wherein between 10ml and 50ml of foam is injected in a single injection, preferably between 10ml and 40ml, more preferably between 15ml and 30ml.

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1. A method for phlebologic treatment of a patient, the method comprising having the patient breathe oxygen or an oxygen enriched atmosphere for a predetermined period prior to injecting a foam,

wherein the foam consists of a liquid phase and a gas phase,

wherein the liquid phase comprises a sclerosing agent and the gas phase consists of 0.01% to 0.6% by volume gaseous nitrogen, the balance being other physiologically acceptable gas chosen from oxygen, carbon dioxide and a mixture thereof,

wherein substantially the entire greater saphenous vein of one leg of a human patient is treated by a single injection of foam, and

wherein between 15ml and 30ml of foam is injected in a single injection.

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